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Tailoring the DNA SAM surface density on different surface crystallographic features using potential assisted thiol exchange

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ABSTRACT

The influence of surface crystallography and applied potential on the thiol-exchange procedure to create mixed alkylthiol DNA SAMs is detailed. A single crystal gold bead and fluorophore labeled thiol modified DNA were used to characterize the resulting surface modifications. The thiol-exchange occurs with different efficiencies on the low index planes (111,100,110) as compared to 311 and 210. Positive applied potentials (>0/SCE) result in 10 × higher coverage than when compared to deposition at the open circuit potential (OCP) over the same 60 min time period. Negative potentials (< 0/SCE) resulted in less uniform coverage with the 111 facet being significantly modified. The electrolyte used during the deposition was a 10 mM TRIS Buffer with 100 mM NaCl 500 mM $MgCl_2$. The influence of [Cl^-] was studied showing it had a significant impact on the thiol-exchange at the positive potentials, where higher [Cl^-] resulted in higher DNA coverages and a more uniform coverage across the multi-crystalline surface. The local environment of the thiol-exchanged DNA SAMs were compared for different regions on the surface using potential driven DNA reorientation modulating the fluorescence intensity. These results showed a common behaviour from all surfaces suggesting that the DNA SAMs prepared by thiol-exchange were consistently prepared with a variable surface concentration controlled by potential and time.

1. Introduction

Self-assembled monolayers(SAMs) of DNA on gold surfaces are used in the manufacturing of a variety of nucleic acid based biosensors (e.g., aptamer-based biosensors) [1–4]. They are conventionally made by exposing a clean gold surface to thiol-modified DNA molecules which chemisorb to the gold surface via a gold-thiol covalent interaction. In a consecutive step, non-specifically adsorbed DNA, or DNA adsorbed to the gold surface via the nitrogenous bases, is displaced with exposure to a short chain alkylthiol [5,6]. Typically, this process takes place on a gold surface that is at the open circuit potential(OCP). However, the surface coverage and local environment around the DNA in a SAM are not easily controlled when manufacturing these multicomponent SAMs and are known to impact biosensor performance. Thus the ability to easily tailor the formation of a DNA SAM and control over its local environment is desirable. Control over the spacing between DNA molecules is needed to ensure the surface is easily accessible for analytes, thereby requiring a homogeneously modified surface. At the same time a sufficiently large number of adsorbed DNA molecules are needed to ensure high sensitivity[7,8]. One approach was the creation of nanostructures via electrodeposition on planar electrode surfaces which increases DNA surface coverage while maintaining accessibility [9–11].

Many strategies have been detailed for controlling the DNA surface coverage in SAMs such as increasing the immersion time or the concentration of the DNA in the immobilization buffer(IB) [5]. Increasing the ionic strength of IB can increase the DNA coverage by shielding the electrostatic repulsion between the adsorbed DNA [7]. Typically, mercaptohexanol(MCH) is used to displace non-specifically adsorbed DNA [5–7] though not completely effective [12,13]. An alternative approach was used where thiolated DNA displaces or competes (e.g., thiol-exchange) with a preformed MCH SAM which resulted in less physisorbed (non-specifically adsorbed)

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DNA as well as a decreased DNA coverage [14]. Also performed was a coadsorption approach where both DNA and the alkylthiol are present in the deposition solution and competitively adsorb on the initially clean gold surface [12,15,16]. A reductive desorption strategy has been used to decrease DNA coverage via negative potential pulses in the presence of a short alkylthiol (MCH) [8]. In these examples, the DNA self-assembly step proceeded at OCP or without control over the substrate potential.

Chemisorption of thiols to the gold surface can be considered an oxidative process and application of positive potentials during deposition has shown promise for enhancing the adsorption of thiols and controlling DNA coverage [17–20]. Ma and Lennox showed that preparing a mixed-composition SAM via alkanethiol deposition on gold while applying constant positive potentials resulted in faster adsorption kinetics and far fewer defects as compared to assembly at OCP [21]. Similar results were reported when depositing thiol-modified DNA on gold [7]. More recently, Jambrec and colleagues demonstrated pulsing the potential on a bare gold surface in a solution of thiolated DNA led to faster DNA monolayer formation [22], when compared to a constant potential explained as due to a kinetic stirring effect from the pulsed potential [23].

Potential-controlled (or assisted) methods have demonstrated that increasing the DNA surface coverage is possible, but the uniformity of the DNA coverage on gold surfaces that are multicrystalline or atomically rough has not been addressed. Typically characterization of these DNA SAMs is done via electrochemical and impedance methods, which provide only an average coverage or permeability over the total electrode surface. Additionally, studies of the electrochemically controlled preparation or modification of a DNA SAM has not emphasized study across different surface crystallographies. Since point of care electrochemical biosensors will be prepared using sputtered gold films, the resulting surface is usually polycrystalline with many small grains of various orientations [24]. Since the arrangement and packing of SAMs is influenced by the underlying surface crystallography [25,26], its influence on the DNA SAMs created using applied potentials should be investigated. This can be achieved using a single crystal bead electrode that has well defined arrangements of different surface crystallographic regions on its surface [26]. Moreover, the assembly of DNA SAMs on a gold single crystal bead electrode can be characterized using fluorophore labeled DNA and in-situ electrochemical fluorescence microscopy imaging (iSEFMI). This enables a self-consistent comparison of the amount of DNA SAM on the electrode surface in a specific region that has a designated crystallographic orientation [26,27]. Using this technique, a comparison of the SAMs made via the DNA/MCH displacement process and those by the MCH/DNA thiol exchange process proposed by Murphy and colleagues [14] was evaluated. A significant variation of DNA coverage for different crystallographies [26] was observed, more so in the thiol-exchange method.

Described herein is progress towards the creation of a homogenous and reproducible DNA SAM using the thiol-exchange method through the application of a potential during the DNA deposition step (schematically outlined in Fig. 1). A MCH SAM modified gold bead electrode was prepared. It was then immersed in an IB containing thiolated DNA and a potential was applied for up to an hour. The resulting modified surface was analyzed using iSEFMI so as to evaluate the extent of DNA thiol-exchange on specific crystallographic regions in addition to characterizing the local environment of the tethered DNA. The use of single crystal gold bead electrodes enables a direct comparison of the assembly on surfaces that have different crystallographies in a self-consistent manner and allows measurement of relative DNA coverage. A comparison of SAMs prepared via thiol-exchange at positive and negative applied potentials (vs. SCE) with SAMs made at OCP is presented in addition to investigating the influence of electrolyte composition.

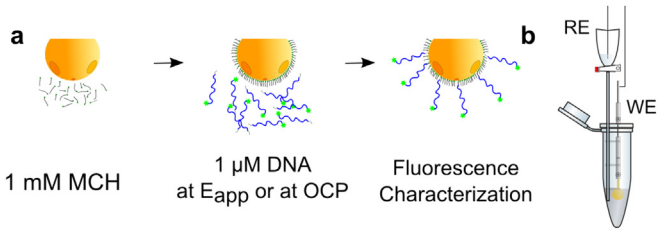

Fig. 1. a) Schematic representation of the preparation of a MCH/DNA SAM via thiol exchange. A single crystal gold bead electrode is modified creating a MCH SAM; it is immersed in a 1 μM solution of thiol modified DNA which is fluorophore labeled. The deposition proceeds at OCP or with an applied potential. b) The potential is applied or measured using a two electrode arrangement in an eppendorf tube.

2. Experimental

2.1. Materials

Mono-crystalline gold bead electrodes were made from 99.999% pure gold wire (1 mm dia., Alfa Aesar) as described previously [26]. 6-Mercapto-1-hexanol, (99% Sigma Aldrich) was diluted to 1 mM in MeOH (HPLC grade, Fisher) for immediate use from a 50 mM stock solution which was stored in an eppendorf tube (1.5 mL siliconized) at −20 °C and renewed every month. The single stranded DNA molecules (Integrated DNA Technologies) were composed of 30 bases with $HO(CH_2)_6SS(CH_2)_6$ at the 5′ end and the 3′ end labeled with AlexaFluor®488. Prior to use, the disulphide was reduced using tris(2-carboxyethyl)phosphine-hydrochloride (>98% Sigma Aldrich) with KOH (99.99% semiconductor grade Sigma Aldrich) and filtered using a GE Microspin™ G-50 column as described previously [14]. The sequence (5′CTG TAT TGA GTT GTA TCG TGT GGT GTA TTT 3′) was selected so as to be not self complementary and incapable of making secondary structures (confirmed with Oligiocalc, DNA Melt and mfold) [14,26]. DNA was stored as 5 μM solutions in 10 mM TRIS(2-amino-2-(hydroxymethyl)propane-1,3-diol) buffer (containing TRIS Base and TRIS HCl, Bioperformance >99.0%, Sigma Aldrich for both), 100 mM NaCl (≥99.5% BioXtra, Sigma Aldrich) pH 7.5 buffer in a sealed eppendorf tube at −20 °C for up to 2 weeks after reduction of the disulphide. The TRIS buffer is used to inhibit DNAse enzymes and prevent DNA denaturation [28]. Immobilization onto the gold electrode was performed in a DNA solution which was diluted to 1 μM in a pH 7.5 IB containing 10 mM TRIS 100 mM NaCl and 500 mM $MgCl_2$ (>99% Sigma Aldrich). The DNA surface concentration was measured for select surfaces using ruthenium (III) hexaammine chloride (99%, Sigma Aldrich) which was stored as 10 mM solutions in water and bubbled with Ar prior to long term storage at −20 °C. All aqueous solutions were prepared in Millipore water (>18.3 MΩ cm, MilliQ system).

2.2. Au electrode preparation and modification

Single crystal gold bead electrodes were flame annealed and cleaned prior to modification as described previously [26]. The cleaned Au electrodes were rinsed with Millipore water and then immersed in a solution of 1 mM MCH in MeOH for 30 min. The MCH SAM coated bead was rinsed and stored in MeOH before same day modification with DNA. Before DNA deposition, the MCH layer was washed with Millipore water and then carefully positioned in an eppendorf tube containing 1 μM of the thiol-modified fluorescently labeled DNA in the IB (80 μL). Also inserted into the tube was a glass pipette connected to a stopcock which acted as the salt bridge to a